

Supplemental material

A multi-center evaluation of a sequence-based protocol to subtype Shiga toxins and standardize Stx nomenclature

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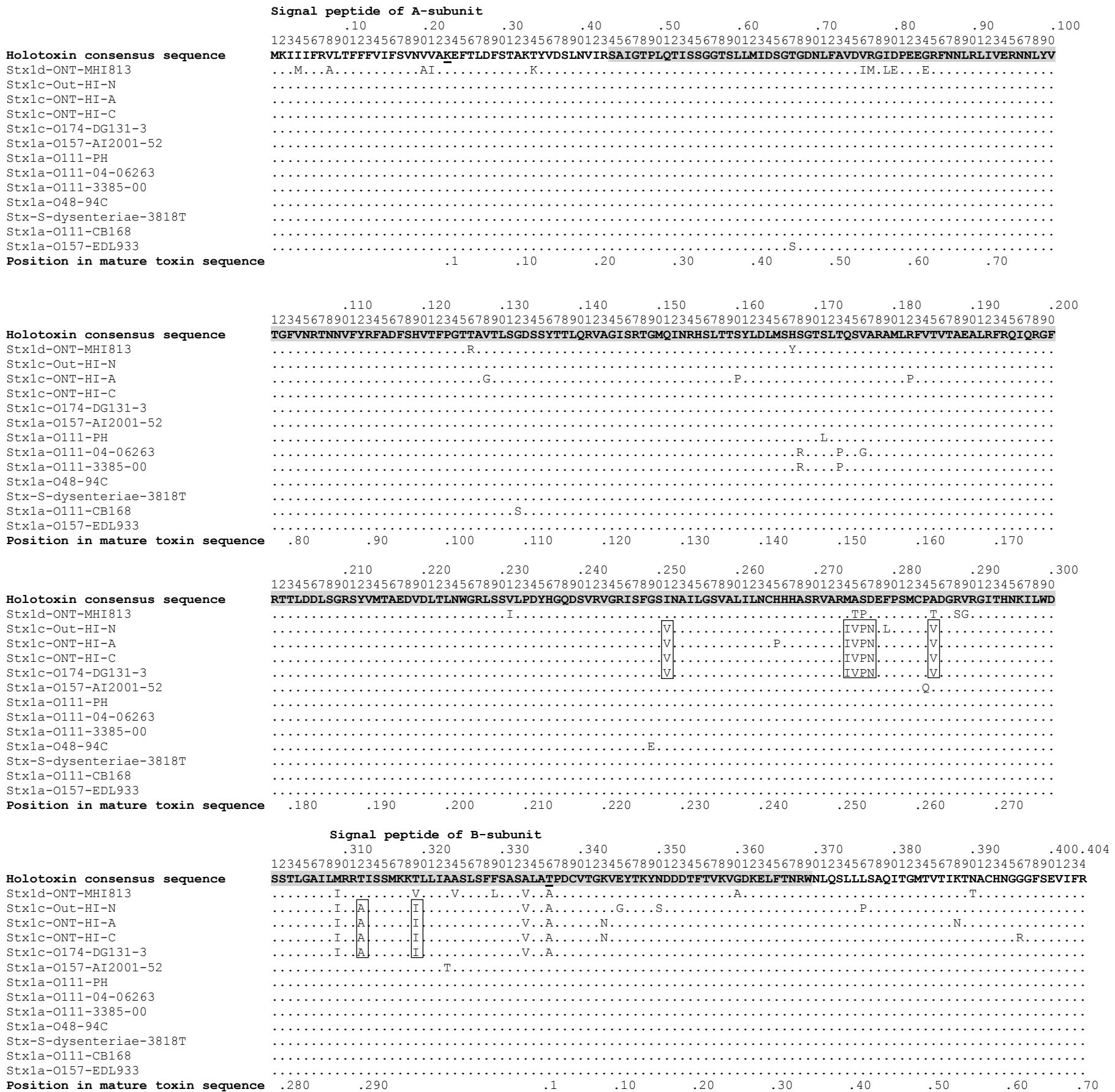


Figure S1. Amino acid sequences of the complete Stx/Stx1 holotoxins (top numbers) including the signal peptide regions and the complete A- and B-subunit and mature toxins (bottom numbers) of the 13 subtype variants of Stx/Stx1.
Box: Motifs that seem to define Stx1c.

Box: Motifs that seem to define Stx1c. Shaded ^ sequence indicates the start.

Shaded AA sequence indicates the sequence covered by partial sequencing as described in Materials & Methods. The first AA in the mature toxin subunits A and B are underlined.

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Figure S2. Amino acid sequences of the complete Stx2 holotoxins (top numbers) including the signal peptide regions and the complete A- and B-subunits and mature toxins (bottom numbers) of the 93 variants within the 7 subtypes of Stx2. Serine (**S**) at position 291 and glutamic acid (**E**) at position 297 in the mature toxin defining the activatable tail are underlined. Subtypes Stx2b, Stx2g and 4 variants Stx2e share these two amino acids but differ either in the C-terminal end of the A₂-subunit (Stx2b) or in the B-subunit (4 variants of Stx2e and Stx2g). Box: Motifs that combined could determine the activatable property of Stx2d.

*: Minor single substitutions in one or more variants within each of the 7 subtypes.

Shaded AA sequence indicates the sequence covered by partial sequencing as described in Materials & Methods.

The first AA in the mature holotoxin consensus sequence of subunits A and B are underlined.

- a) Stx2b variants comprise two subgroups with 4 variants (Stx2b-ONT-I7606, Stx2b-O111-PH, Stx2b-O174-031 and Stx2b-O128-24196-97) in one group and the other 12 variants in the other group.
 - b) Includes Stx2e-O8FHI-1106-1092 on the stretch from position 100 to 199
 - c) A subgroup of three Stx2a variants (Stx2a-O113-CL-3, Stx2a-O104-G5506 and Stx2a-O8-VTB178) differs from 18 other Stx2a variants by S, E and D in positions 313, 319 and 362.
 - d) Only four Stx2e variants (Stx2e-OR-TS09-07, Stx2e-O26-R107, Stx2e-O139-S1191 and Stx2e-O101-E-D43) have the S in position 313.
 - e) Six Stx2b variants lack the last two AAs ND.

Table S1. Number of nucleotides and amino acids in the A subunits, intergenic region sequences and B subunits in subtypes of Shiga toxin, Shiga toxin 1 and Shiga toxin 2. Intergenic sequences were not submitted for *stx_{2c}*-O157-020324 (Acc. No. AY739670 & AY739671) and *stx_{2d}*-C-freundii-LM76 (Acc. No. AY739670 & AY739671).

Toxin subtype	A subunit nucleotides (Amino acids)	Intergenic region No. of nucleotides sequence	B subunit nucleotides (Amino acids)
<i>stx</i> , <i>stx_{1a}</i> & <i>stx_{1d}</i>	948 (315)	9 gggggtaaa	270 (89)
<i>stx_{1c}</i>	948 (315)	10 ggggggtaaa ^a	270 (89)
<i>stx_{2a}</i> , <i>stx_{2c}</i> & <i>stx_{2d}</i> ^b	960 (319)	11 aggagttaaag ^c aggtgataagc ^d	270 (89)
<i>stx_{2b}</i>	960 (319)	12 caggagttaaat ^e ctggagttaaat ^f cgggagttaaat ^g	264 (87)
<i>stx_{2e}</i>	960 (319)	12 aaggagttaga ^e	264 (87)
<i>stx_{2f}</i>	960 (319)	12 caggggggtgaat	264 (87)
<i>stx_{2g}</i>	960 (319)	12 aaggagttaaagc ^h aaggagttaaat ⁱ	270 (89)

^a Only two of the four analyzed sequences contain the intergenic region.

^b *stx_{2d}*-C-freundii-LM76 has an insertion of aat at positions 190-192 resulting in a total of 963 bp in the A subunit;

^c t: 13 *stx_{2a}*, 16 *stx_{2c}* and 16 *stx_{2d}* variants; c: 8 *stx_{2a}*, 1 *stx_{2c}* (*stx_{2c}*-O157-E32511) and 1 *stx_{2d}* (*stx_{2d}*-O22-KY-O19) variants. One *stx_{2c}* (*stx_{2c}*-O157-020324) did not contain the intergenic region.

^d *stx_{2a}*-ONT-EBC210

^e 14 variants

^f *stx_{2b}*-O8-S-9

^g *stx_{2b}*-O118-EH250

^h 3 variants

ⁱ *stx_{2g}*-Out-S-8

Table S2. Designations of prototype Stx toxins ^a, and previous designations of genes and/or toxins.

Table adapted and revised from (59) and (60).

Toxin subtype ^b Stx	Toxin variant designation	Prototype Organisms. Strains used for validation of the subtyping protocol are shown in bold (reference strains for each subtype are <u>underlined</u>)	Serotype of prototype organism. ^c Bold: Strains serotyped as part of this study	GenBank accession No. (reference)	Previous designation or synonym for toxin gene. ^d	Previous designation or synonym for toxin
Stx ^e	Stx-S-dysenteriae-3818T ^f	<u>3818T</u> (66) CB7888 (9)	<i>S. dysenteriae</i> <i>S. sonnei</i> [H16]	M19437 (66) AJ132761 (9)	<i>stx1</i>	<i>Stx1</i>
Stx1a	Stx1a-O157-EDL933	<u>EDL933</u> (47)	O157:H7	M19473 (28)	<i>slt-I</i>	SLT-I
		H-19B (35)	O26:H11	M16625 (13)		VT1
		H30 (35)	O26:H11	M23980 (36)		VT1
	Stx1a-O111-04-06263	04-06263 (76)	O111:NM	AM230663 (76)		
	Stx1a-O111-3385-00	3385-00 (76)	O111:NM	AM230662 (76)	<i>stx1</i>	
	Stx1a-O111-CB168	CB168 (50)	O111:[H8]	Z36900 (50)		SltI/CB168
	Stx1a-O111-PH	PH (53)	O111:[H8]	L04539 (53)		SltI/PH
	Stx1a-O157-AI2001-52	AI2001-52	O157:H7	AB083044 (67)		
	Stx1a-O48-94C	94C (50)	O48:H21	Z36899 (50)		SltI/O48
Stx1c	Stx1c-O174-DG131-3	<u>DG131/3</u> (33,50)	O174:H8	Z36901 (33,50)	<i>stx1ox3, stx1c</i>	stx(1c)
	Stx1c-ONT-HI-A ^g	#HI-A (2)	ONT:NM	AB071619 & AB071620 (2)		
	Stx1c-ONT-HI-C ^g	#HI-C (2)	ONT:H34	AB071623 & AB071624 (2)		
	Stx1c-Out-HI-N	#HI-N (3)	Ount:H-	AB048237 (3)		
Stx1d	Stx1d-O8-MHI813 ^h	<u>MHI813</u> (11)	O8:K85ab:HR	AY170851 (11)	<i>stx1d</i>	

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Stx2a	Stx2a-O157-EDL933	<u>EDL933</u> (47)	O157:H7	X07865 (27)	<i>stx2 slt-II</i>	SLT-II
	Stx2a-O48-94C	94C (51)	O48:H21	Z37725 (51)	<i>stx2</i>	SLT-II/O48
	Stx2a-O157-SF-258-98	258/98 (10)	O157:[H7]	AF524944 (10)	<i>stx2</i>	
	Stx2a-O26-126814	126814 (5)	O26:H11	AJ272135 (5)	<i>stx2</i>	
	Stx2a-O26-FD930	FD930 (73)	O26:H4	AF461173 (73)		
	Stx2a-O165-VTB60 ⁱ	VTB60 (44)	O165:H25	AY633471 (44)	<i>stx2</i>	stx2vhd
	Stx2a-O157-A397 ⁱ	A397 (44)	O157:H7	AY633472 (44)	<i>stx2</i>	
	Stx2a-O157-93-111	93-111 (37)	O157:H7	EF441599 (37)		
	Stx2a-O111-928-91	928/91 (37)	O111:H-	EF441609 (37)		
	Stx2a-O101-EBC201 ^j	EBC201 (14)	O101:[H10]	AY443052 (14)	<i>stx2</i>	
	Stx2a-O22-EBC217	EBC217 (14)	O22:H8	AY443054 (14)	<i>stx1 + stx2</i>	
	Stx2a-ONT-EBC210	EBC210 (14)	NT; Strain lost	AY443057 (14)		
	Stx2a-O157-I6581	I6581 (37)	O157:H7	EF441613 (37)		
	Stx2a-E-cloacae-95MV2	95MV2 (52)		Z50754 (52)		
	Stx2a-Acinetobacter-haemolyticus-DS9B	DS9B (21)		DQ344636 (21)		
	Stx2a-O104-G5506	G5506 (37)	O104:H21	EF441619 (37)	<i>stx2</i>	
	Stx2a-O8-VTB178	VTB178 (44)	O8:H19	AY633459 (44)	<i>stx2</i>	
	Stx2a-ONT-23765	23765 (72)	ONT:H2	GQ429162 (72)		

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	Stx2a-O178-TS22-08	TS22-08 (64)	O178:H19	FM998856 (64)		
	Stx2a-O113-TS17-08	TS17-08 (64)	O113:H21	FM998851 (64)		
	Stx2a-O113-CL-3	CL-3 (37)	O113:H21	EF441618 (37)		
Stx2b	Stx2b-O174-031	<u>031</u> (54)	O174:H21	X65949 (54)	<i>stx_{2v}OX392</i> ^k	SLT-II/OX3a / VT2d-OX3a Stx2d-OX3a ¹
	Stx2b-O111-PH (not detected?)	<u>PH</u> (51)	O111:[H8 defective]	L11078 (55)	<i>stx_{2v}O111</i> <i>stx2</i>	SLT-II/O111 / VT2d-O111 Stx2d-O111 ¹
	Stx2b-O118-EH250	<u>EH250</u> (57)	O118:H12	AF043627 (57)		Stx2d/VT2d-Ount ¹
	Stx2b-O22-3143-97	<u>3143/97</u> (58)	O22:H8	AJ313015 (58)	<i>stx_{2d}</i>	
	Stx2b-O40-5293-98	<u>5293/98</u> (75)	O40:H8	AJ567997 (75)	<i>stx2d</i>	
	Stx2b-O96-S-6	#S-6 (1)	O96:H-	AB048225 (1)		
	Stx2b-O111-S-3	#S-3 (1)	O111:H45	AB048223 (1)		
	Stx2b-O128-24196/97 ^m	<u>24196/97</u> (75)	O128:H2	AJ567995 (75)	Stx2d	
	Stx2b-O111-S-1	#S-1 (1)	O111:H45	AB012101 (1)		
	Stx2b-O96-S-7	#S-7 (1)	O96:H-	AB048226 (1)		
	Stx2b-O96-S-10	#S-10 (3)	O96:H-	AB048229 (3)		
	Stx2b-Out-HI-N	#HI-N (3)	Out:H-	AB048238 (3)		
	Stx2b-O8-S-9	#S-9 (3)	O8:H7	AB048228 (3)		
	Stx2b-O93-S-5	#S-5 (1)	O93:H-	AB048224 (1)		

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	Stx2b-Out-S-4	#S-4 (1)	Out:H45	AB012102 (1)		
	Stx2b-ONT-I7606	I7606 (37)	NT	EF441616 (37)		
Stx2c	Stx2c-O157-E32511	E32511 (63)	O157:[H7]	M59432	<i>stx2/stx2c</i> <i>slt-IIc</i>	Stx2v/VT2v SLTIIc
	Stx2c-O157-FLY16	FLY16 (26)	O157:H-	AB015057	<i>stx2</i>	VT2v(pKTN1050)=Stx2vh ⁿ
	Stx2c-O157-C394-03	C394-03 (56)	O157:[H7 defective]	DQ235774 (56)		
	Stx2c-O174-031	<u>031</u> (55)	O174:H21	L11079 (55)	<i>stx2vOX393</i> ^k	SLT-II/OX3b/2 / VT2d-OX3/2 Stx2-OX3b ¹
	Stx2c-O157-469	469 (44)	O157:H7	AY633473 (44)		
	Stx2c-O171-EBC287 ^o	EBC287 (14)	O171:K49:H2	AY443045 (14)	<i>stx2</i>	
	Stx2c-O157-310	310 (44)	O157:H7	AY633467 (44)	<i>stx2</i>	
	Stx2c-O157-A75	A75 (44)	O157:H7	AY633453 (44)	<i>stx1 + stx2</i>	
	Stx2c-O157-Y350-1	Y350-1 (49)	O157:H7	AF291819		
	Stx2c-O157-G5101	G5101 (37)	O157 :H7	EF441604 (37)		
	Stx2c-O157-020324	020324	O157:H7	AY739670 & AY739671 (74)		
	Stx2c-ONT-pVTEC9	pVTEC9 (14)	OR:K+:H-	AY443044 (14)	<i>stx2</i>	
	Stx2c-ONT-EBC219	EBC219 (14)	O?:K17:H7	AY443043 (14)		

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Stx2c	Stx2c-ONT-EBC289	EBC289 (14)	NT	AY443049 (14)		
	Stx2c-O157-V20	V20 (24)	O157:H7	AB071845 (24)	<i>stx</i> _{2vhd}	
	stx2c-OR-TS27-08	TS27-08 (64)	OR:NM	FM998860 (64)		
	stx2c-O177-CB7126	CB7126 (65)	O177:NM	FM177471 (65)		
	Stx2c-O177-06-5121 ^p	06-5121 (19)	O177:[H25]	EU086525 (19)	<i>stx2c</i>	
Stx2d ¹	Stx2d1-O91-B2F1	B2F1 (25)	O91:H21	AF479828	<i>stx2vha/stx2vha</i> <i>stx</i> _{2d1} / <i>stx2d1</i> <i>stx2ha</i> <i>stx2da</i> <i>stx2v-ha</i>	SLT-IIvh/ SLT-IIvh Stx2vha/VT2vha Stx2d1/VT2d1 Stx2vh-a/VT2vh-a VT2v-a
	Stx2d2-O91-B2F1	B2F1 (25)	O91:H21	AF479829	<i>stx2vhb/stx2vhb</i> <i>stx</i> _{2d2} / <i>stx2d2</i> <i>stx2hb</i> <i>stx2db</i> <i>stx2v-hb</i>	Stx2vhb/VT2vhb Stx2vh-b/VT2vh-b Stx2d1/VT2d1 VT2v-b SLT-IIvhb
	Stx2d-O28-MT71	MT 71 (29)	O28ab:H9	AY095209 (29)	<i>stx2c2</i>	Stx2c2
	Stx2d-O157-7279 (VCA negative!)	7279 (43)	O157:H7	X61283	<i>stx2vhc</i> (<i>stx2d3</i>)	Stx2vhc
Stx2d	Stx2d-O8-C466-01B	C466-01B (56)	O8:H19	DQ235775 (56)		
	Stx2d-O174-EC173b ^q	EC173b (20)	O174:H21	AF500190 (20)	<i>stx2d, stx2Bvhb</i>	

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Stx2d	Stx2d -O174-EC1720a ^q	EC1720a (20)	O174:H21	AF500189 (20)	<i>stx2d, stx2Bvha</i>	Stx2cf
	Stx2d-O165-EC1871a	EC1871a (20)	O165:H11	AF500191 (20)	<i>stx2d, stx2Bvhb</i>	
	Stx2d-ONT-EBC275 ^q	EBC275 (14)	O2:H29	AY443047 (14)		
	Stx2d-O103-pVTEC7	pVTEC7 (14)	OR:K+:H48	AY443048 (14)		
	Stx2d-C-freundii-LM76 ^r	LM76 (61)		X67514 & X67515 ^g	<i>C. freundii</i> slt-IIcA and slt-IIcB	
	Stx2d-O73-C165-02	C165-02 (56)	O73:H18	DQ059012 (56)		
	Stx2d-O22-KY-O19	KY-O19 (40)	O22:H-	SNS ^s		
	Stx2d-O6-NV206	NV206 (6)	O6:H10	AF329817		
	Stx2d-O2-EC604a	EC604a (20)	O2:H29	AF500192 (20)		SLT-IIv SLT-IIva ^u SLTIIe/VTe VT2vp
	Stx2d-O113-TS21-08	TS21-08 (64)	O113:H21	FM998855 (64)		
	Stx2d-O113-TS03-07	TS03-07 (64)	O113:H21	FM998840 (64)		
	Stx2d-O55-5905	5905 (37)	O55:[H7]	EF441605 (37)		
Stx2e	Stx2e-O139-S1191	<u>S1191</u> (71)	O139:K12:H1 ^t	M21534 ^t (71)	<i>slt-IIv</i> <i>slt-IIva</i> <i>slt-IIe</i>	SLT-IIv SLT-IIva ^u SLTIIe/VTe VT2vp
	Stx2e-O8-3615-99	3615-99 (58)	O8:H10	AJ313016	<i>stx2e-ONT-26725-97 (75)</i> ^v <i>slt-IIera</i>	VT2vp1

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	Stx2e-O101-E-D42	E-D42 (16)	O101:H-	X81418 (16)	<i>stx2e</i>	
	Stx2e-O101-E-D43	E-D43 (16)	O101:[H9]	X81417 (16)	<i>stx2e</i>	
	Stx2e-O101-E-D53	E-D53 (16)	O101:[H9]	X81416 (16)	<i>stx2e</i>	
	Stx2e-O101-E-D68	E-D68 (16)	O101:[H9]	X81415 (16)	<i>stx2e</i>	
	Stx2e-O26-R107	R107 (32)	O26:H?	U72191		Stx2era
	Stx2e-ONT-2771	2771 (45)	OR:H-	AJ249351 (45)		
	Stx2e-O121-NP9621	NP9621 (70)	O121:HNT	AY332411 (70)		
	Stx2e-O100-TS01-07	TS01-07 (64)	O100:H-	FM998838 (64)		
	Stx2e-ONT-TS03-08	TS03-08 (64)	ONT:H-	FM998846 (64)		
	Stx2e-OR-TS09-07	TS09-07 (64)	OR:H19	FM998844 (64)		
	Stx2e-ONT-TS29-08	TS29-08 (64)	ONT:NM	FN182286 (64)		
	Stx2e-O8-FHI-1106-1092 (VCA negative!)	FHI-1106-1092	O8:H2^w	AM904726 (41)		
Stx2f ^x	Stx2f-O128-T4-97	T4/97 (62)	O128ac:[H2]	AJ010730 (62)	<i>stx2f</i>	
	Stx2f-O89-HI8	H.I.8. (17)	O89:[H2]	M29153 ^y	<i>stx2ev/stx2ev</i> <i>slt-IIvhc</i> <i>slt-IIId</i> ^l <i>stx2f</i>	Stx2ev/ VT2ev/ VTev Stx2vp2/VT2vp2 Stx2va/ Stx2v SLTIIvhc SLT-IIId/VT2d ^l SLTIIva

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	Stx2f-O115-F08-101-31	F08-101-31 (15)	O115:NM	AB472687 (15)		
Stx2g	Stx2g-O2-7v	<u>7v</u> (38)	O2:H25	AY286000 (38)	<i>stx2g</i>	
	Stx2g-Out-S-8	#S-8 (3)	Out:H25	AB048227	<i>stx2g</i>	
	Stx2g-O2-S86	S86 (18)	O2:H25	AJ966783	<i>stx2g</i>	
	Stx2g-O2-HI-11	#HI-11 (2)	O2:Hnt	AB048236 (2)		

The 62 reference strains that were referred to the WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella* strain collection are shown in the manuscript **Table 2**, and are available upon request.

^a Data compiled from references (4,11,12,17,25,30,31,33,34,39,40,68,69).

^b Toxin types have traditionally been defined according to antigenic variability, differences in toxicity for tissue culture cells and/or animals, their capacity to be activated by mouse elastase and differences in DNA or amino acid sequences. In this Table the subtypes are defined by phylogenetic analyses described in the text.

^c Serotyping was done independently in two of the participating laboratories. Square brackets around an H type indicates genotypic *fliC* typing by PCR and RFLP analysis of non-motile strains using the method described by Beutin *et al.* (7).

^d Results obtained using the PCR/RFLP *stx* subtyping described by Beutin *et al.* (8) are shown in the manuscript **Table 2**.

^e Stx/*stx* nomenclature is reserved for Shiga toxin from *Shigella* spp. – see principles for the nomenclature of Stx and *stx* genes in the Discussion.

^f The sequence with accession No. X07903 (36) is prior to accession No. M19437 (66) but not complete. It differs by one silent nucleotide in the B sub-unit in *S. sonnei*, strain CB7888, accession No. AJ132761 (9).

^g Only the structural genes for the A and B subunits are given. Thus, the intergenic nucleotide region between the A and B subunit is unknown.

- ^h Stx1d in strain 92-1251 (Acc. No. AB050958), which is serotype O121:H7, is identical to Stx1d-ONT-MHI813 which was published first. It is mentioned here because several biological properties were examined in this study (48) and not examined in strain MHI813 (11). Strain 92-1251 is chosen as reference strain over strain 92-1252 (Acc. No. AB050959) because this strain has now lost the gene. The toxin used for biological activities (Vero cell cytotoxicity, lethal activity in mice and *in vitro* inhibitory effect on protein synthesis) and serology was purified from strain 92-1252 however (48).
- ⁱ Strains VTB60 and A397 were also positive for subtype *stx_{2c}* using the subtyping protocol described in this paper.
- ^j The original gene was cloned from wild type strain EBC201 into plasmid pEHEC400 and published as such. Both strains - **EBC201** and **pEHEC400** (with the cloned gene) - were used in the validation of the subtyping protocol. We have re-sequenced and confirmed the *stx2* gene and serotyped the EBC201 wild type strain and chosen to use this as the reference strain.
- ^k In Table I by Bastian *et al.* (4) (and only here), the designations of these two toxins have been erroneously cited. The correct association between Accession No, publication year and suffix is used here in this Table and as analysed in the rest of figures and text of (4).
- ^l There are several toxins suffixed by “d” in the literature: The Stx2d toxins of O91:H21 (42), the *stx₂-Ogroup/ strain designation and/or year* variants by Paton *et al.*(51,53,54,57) and the SLT-IIId/VT2d (= Stx2f) toxin produced by strain H.I.8. (serotype O128:H2) as proposed by Gyles (22). Stx2-O118/VT2-O118 - formerly known as VT2d-Ount (57) - is expected to be non-activatable based on analysis of the nucleotide sequence (Denis Piérard and Angela Melton-Celsa; unpublished); The original strain has been retyped as O118:H12 (Lothar Beutin and Flemming Scheutz, unpublished). We support the use of the “d” designation for activatable Stx2 toxins as proposed by Melton-Celsa *et al.* (42) and have listed the variants that have been confirmed as activatable by the use of intestinal mouse mucus and the strains that we believe have the potential to be activated.
- ^m Strain 24196/97 is O86:H- in the paper (75), Table 2 and ONT:H- under accession No. AJ567995. It was serotyped by WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella* as serotype O128:H2. The sequence is identical to strain 6451/98, serotype Or:HNT in the paper and O16:H48 under Acc. No. AJ567996. This strain was lost. Furthermore, the serotype of strain 6451/98 suggests that this was a host K-12 strain used to clone the toxin gene. We prefer to use wild type strains in the reference collection.
- ⁿ These two sequences were first published by Lin *et al.*(40) and designations Stx2vhc (Lin) & Stx2vhd were used by Nakao (46). The sequences were however not submitted to GenBank. We found the sequence from cloned plasmid pKTN1050 from strain TK-051 to be identical to Stx2c-O157-FLY16 and have chosen to use FLY16 as the reference strain.
- ^o Strain EBC287 was also positive for subtypes *stx_{2b}* and *stx_{2d}* using the subtyping protocol described in this paper.
- ^p Strain 06-5121 was also positive for subtype *stx_{2d}* using the subtyping protocol described in this paper.
- ^q Strains EBC275, EC173b and EC1720a were also positive for subtype *stx_{2b}* using the subtyping protocol described in this paper.
- ^r The *stx* genes were not stable in the strain and later investigations were not able to perform further work. Neither the original sequences, nor the strain were kept (pers. comm. Herbert Schmidt 27th August 2009).

- ^s SNS: Sequence not submitted to GenBank.
- ^t This sequence is identical to the sequence in strain 412, Accession No M36727 submitted later in 1988 (23). The exact serotype is not given in any of these references but the full serotype has been confirmed to be O139:K12:H1 by The WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella*.
- ^u This designation has also been referred to for the Stx2f toxin produced by strain H.I.8. (serotype O128:H2).
- ^v In our first version this variant was listed as Stx2e-ONT-26725-97 (75). Subsequent analyses showed that Stx2e-O22-3615-99 (58) only differs by two silent nucleotides and that the submission of the sequence is prior (2002) to that of Stx2e-ONT-26725-97 (2005). Subsequently, Stx2e-O22-3615-99 replaces Stx2e-ONT-26725-97 as prototype variant.
- ^w Serotype confirmed on a non-toxin-producing isolate by WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella*.
- ^x The nucleotide sequence of the former *stx2ev* of strain H.I.8. (published as serotype O128:H2) is nearly identical to the recently published *stx2f* found in strain T4/97 (serotype O128:H2) from feral pigeons (62). As its nucleotide sequence is distinctly different from those of the other Stx2 toxins and variants as well as Stx/Stx1, we support the proposal of renaming *stx2ev* as *stx2f*.
- ^y This sequence is identical to the sequence in strain T4/97, serotype O128:H2, Accession No AJ010730 submitted in 2005 (62). M29153 is the first published sequence of *stx2f* but as we have not been able to confirm the O group O128 (several copies from different laboratories were typed), and because *stx2f* seems to be very common in O128 strains (unpublished) we have chosen to use T4/97 as the reference strain.

Table S3. List of reference strains harboring the *stx* gene subtypes, their O:H serotype and additional virulence genes

SSI collection D number	Strain	Control for toxin subtype	Toxin variant designation	GenBank accession No.	Results obtained using the present method	Serotype	Additional virulence genes
D2653	EDL933	Stx1a	Stx1a-O157-EDL933	M19473	<i>stx_{1a}</i> + <i>stx_{2a}</i>	O157:H7	<i>eae, ehxA, astA</i>
D3602	DG131/3	Stx1c	Stx1c-O174-DG131-3	Z36901	<i>stx_{1c}</i> + <i>stx_{2b}</i>	O174:H8	
D3522	MHI813	Stx1d	Stx1d-O8-MHI813	AY170851	<i>stx_{1d}</i>	O8:K85ab:Hrough	<i>eae</i>
D2435	94C	Stx2a	Stx2a-O48-94C	Z37725	<i>stx_{1a}</i> + <i>stx_{2a}</i>	O48:H21	<i>ehxA, saa</i>
D3428	EH250	Stx2b	Stx2b-O118-EH250	AF043627	<i>stx_{2b}</i>	O118:H12	<i>astA</i>
D2587	031	Stx2c	Stx2c-O174-031	L11079	<i>stx_{2b}</i> + <i>stx_{2c}</i>	O174:H21	
D3435	C165-02	Stx2d	Stx2d-O73-C165-02	DQ059012	<i>stx_{2d}</i>	O73:H18	<i>astA</i>
D3648	S1191	Stx2e	Stx2e-O139-S1191	M21534	<i>stx_{2e}</i>	O139:K12:H1	
D3546	T4/97	Stx2f	Stx2f-O128-T4-97	AJ010730	<i>stx_{2f}</i>	O128ac:[H2]	<i>eae, bfpA, astA</i>
D3509	7v	Stx2g	Stx2g-O2-7v	AY286000	<i>stx_{2g}</i>	O2:H25	<i>ehxA, astA, estAp</i>

* May result in both fragments at 179 bp and 280 bp.

The reference strains or DNA lysates of these strains may be obtained from:

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